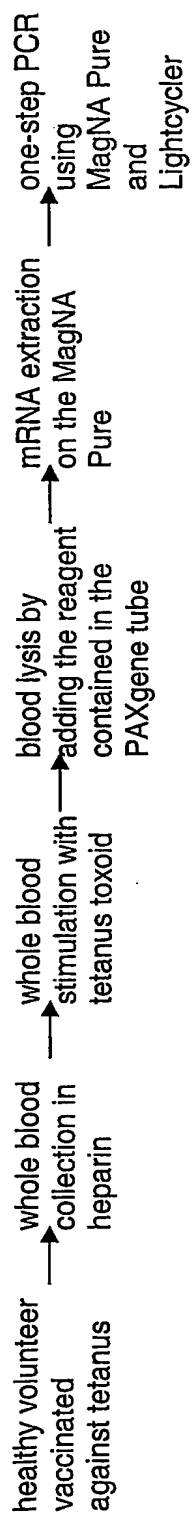


## Figures

Figure 1. Strategies followed in the given examples

Figure 1.1 Ex vivo monitoring of immune response against tetanus toxoid.



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Figure 1.2 Strategy followed in example 3.

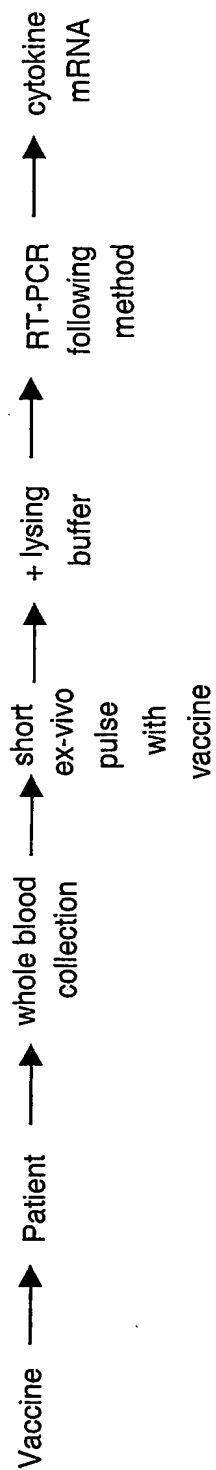


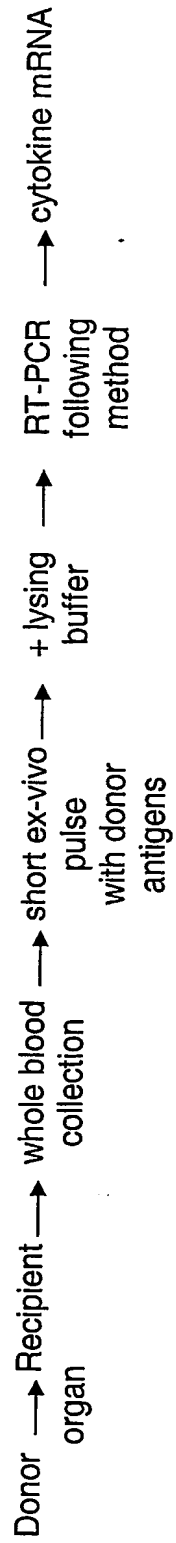
Figure 1.3 Strategy followed in example 4



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Figure 1.4 Strategy followed in example 5.



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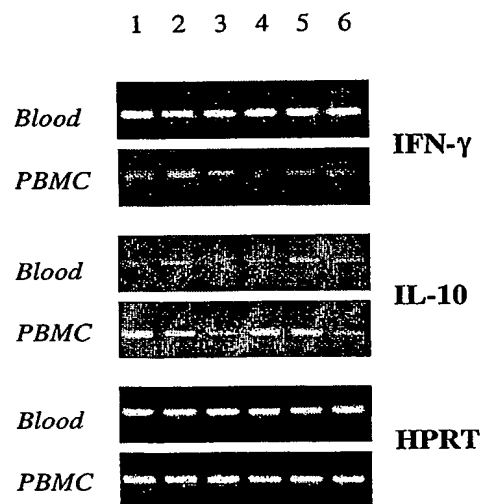


Figure 2.1

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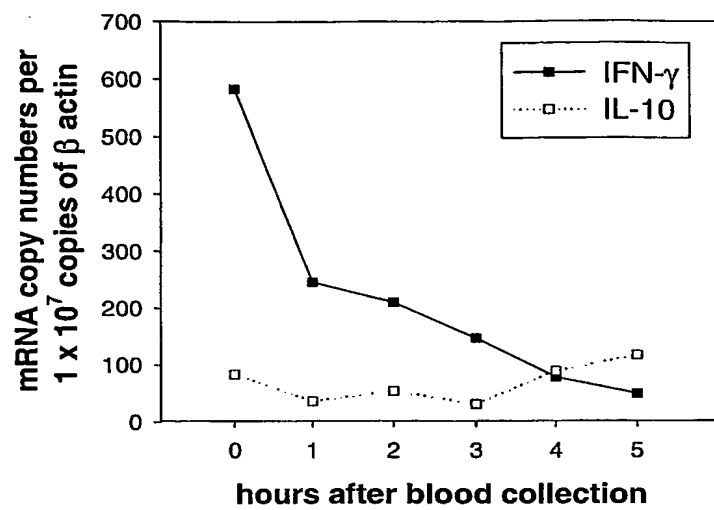
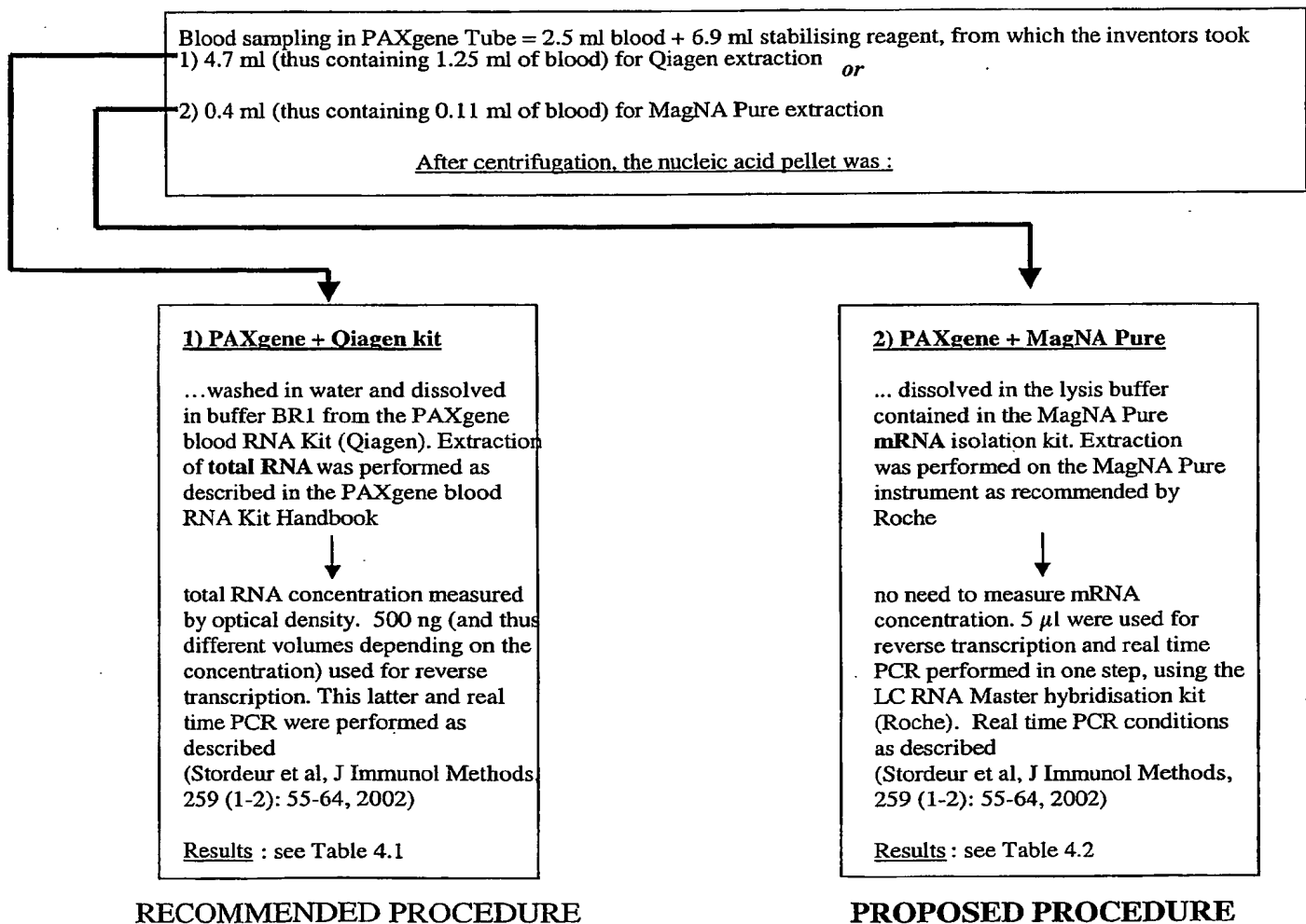


Figure 2.2

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Figure 3:



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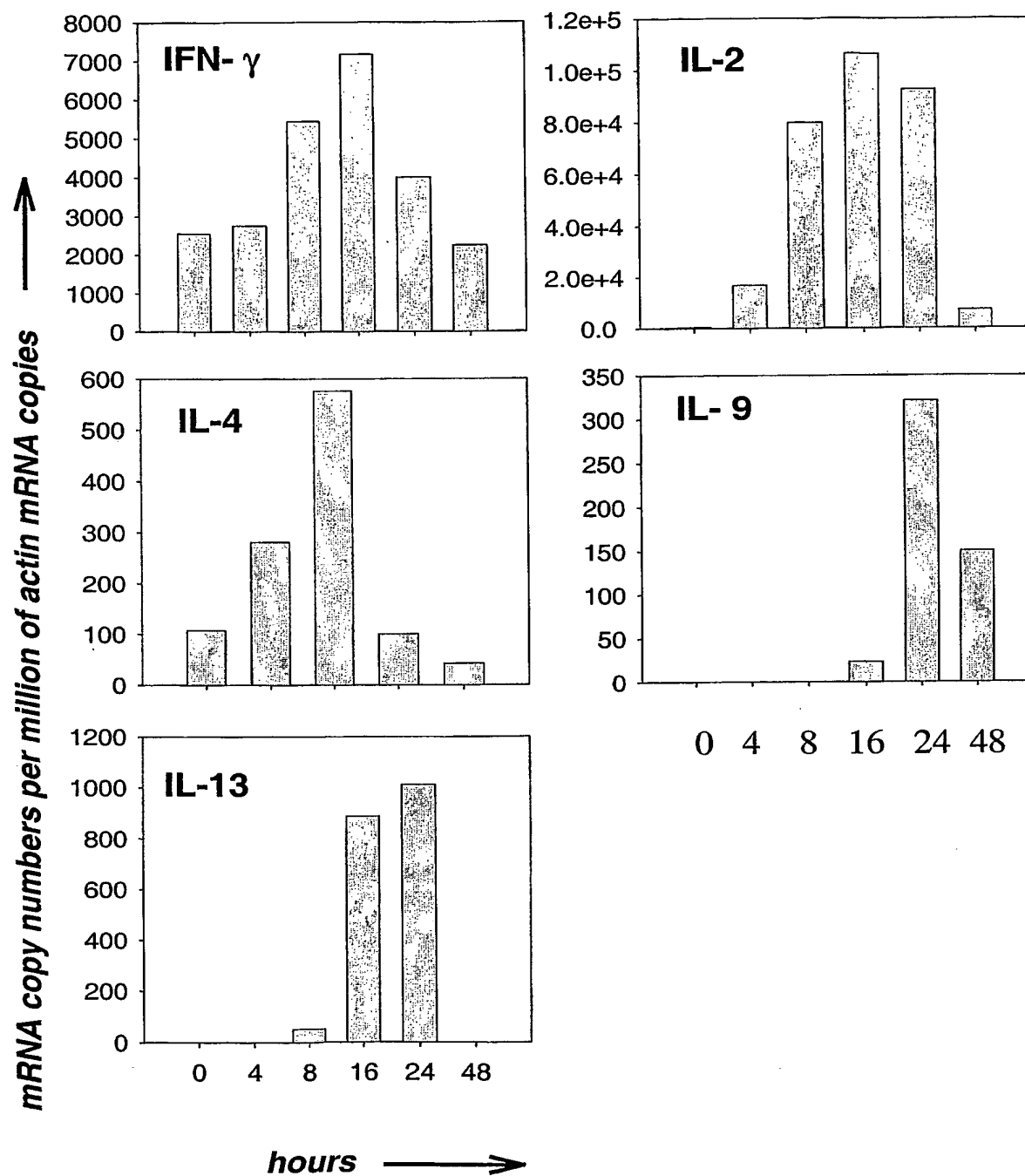


Figure 4



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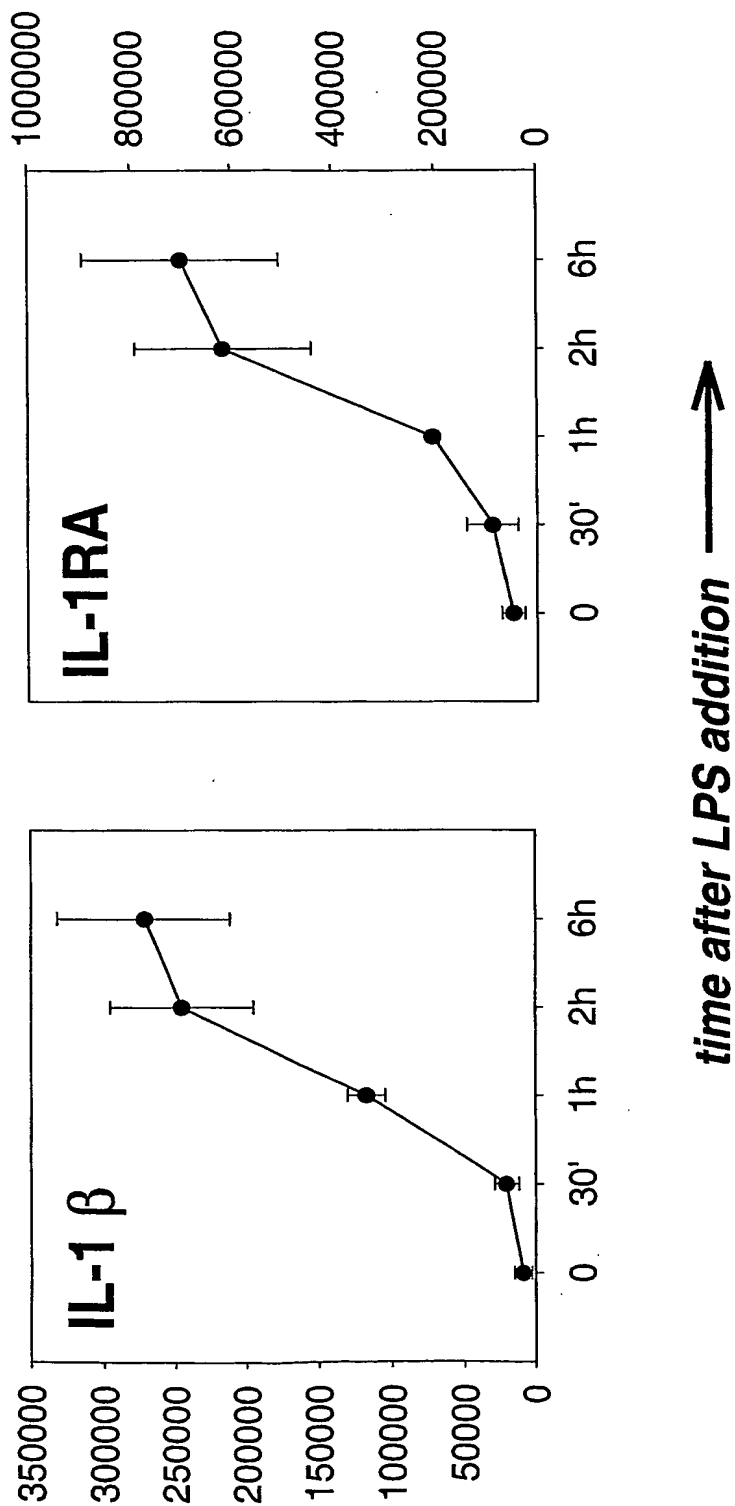


Figure 5

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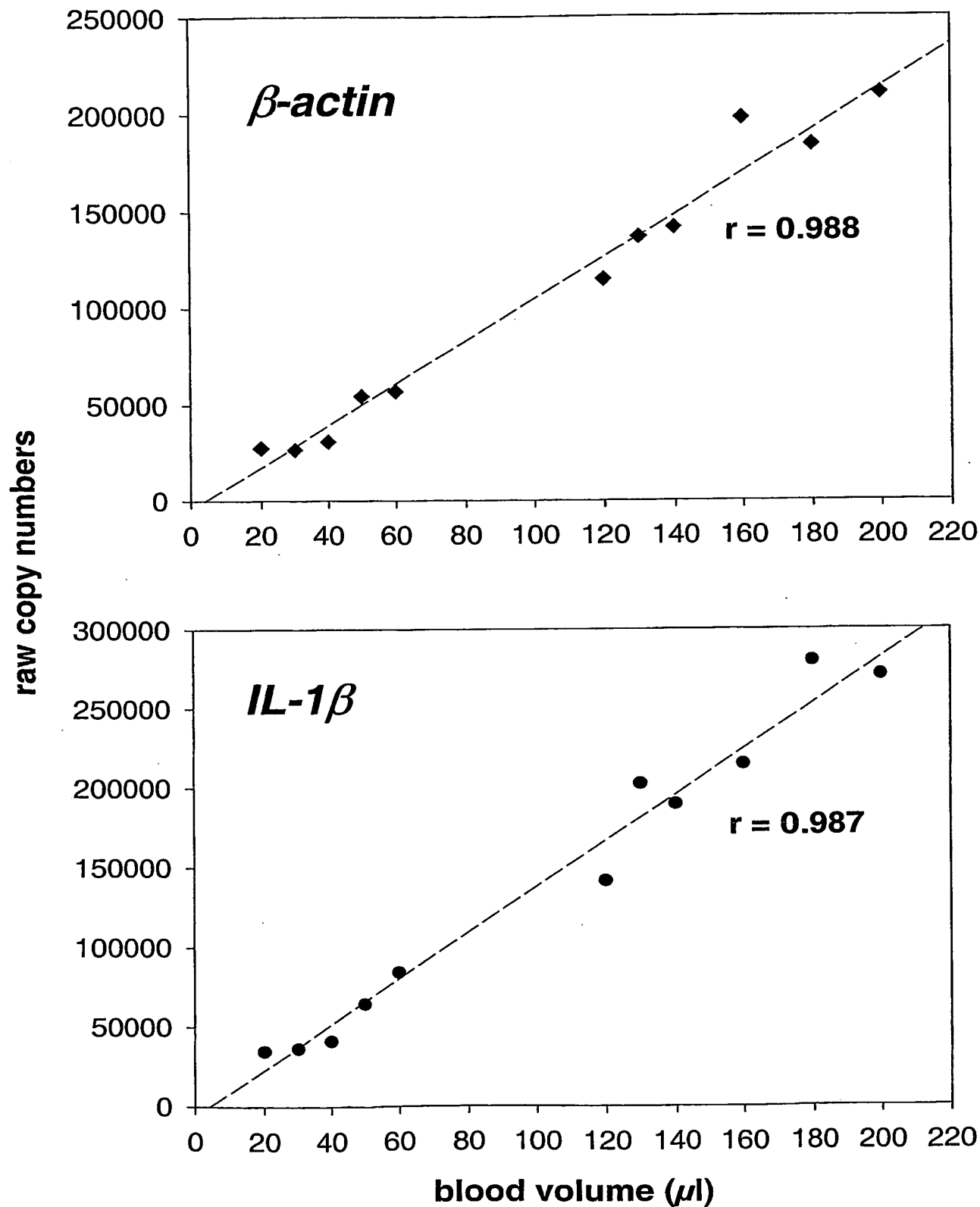
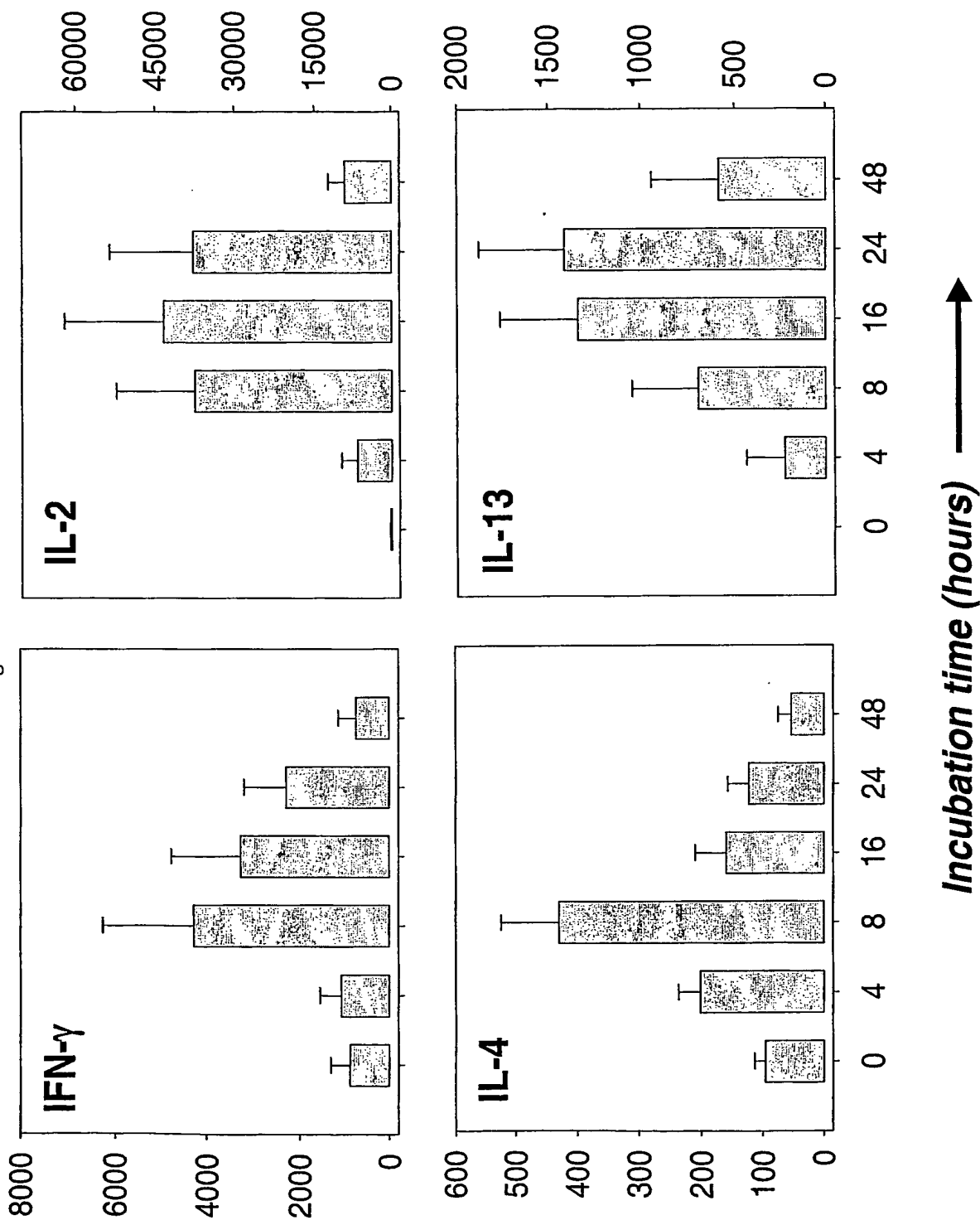


Figure 6

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Figure 7



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Figure 8

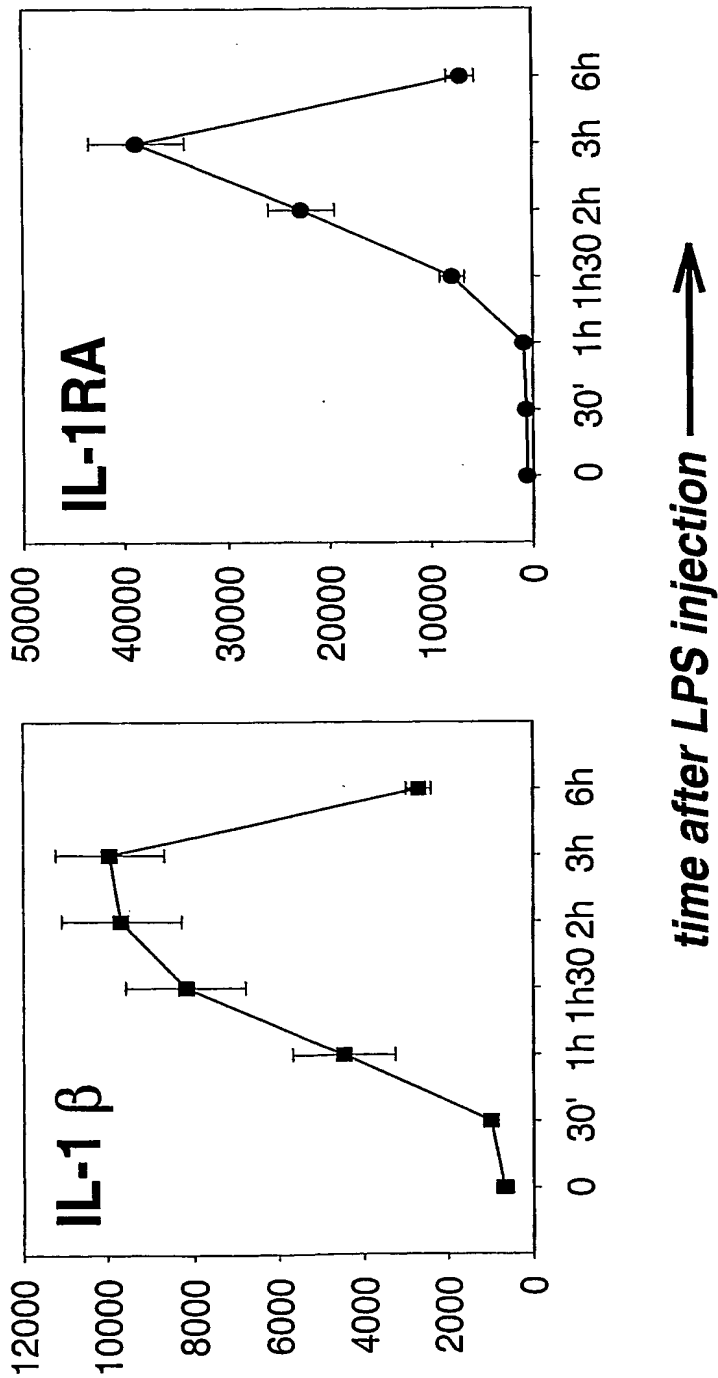
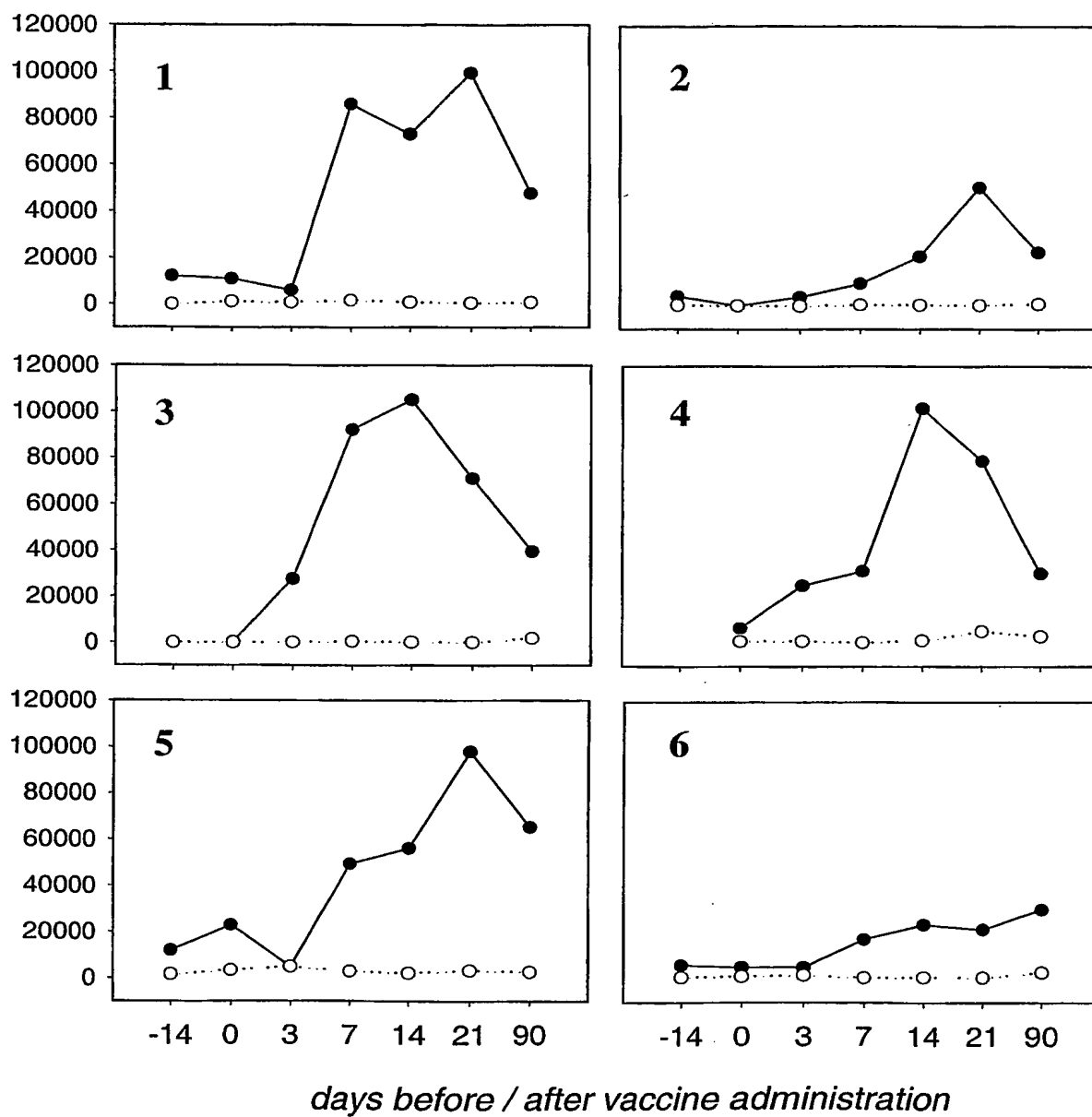


Figure 9



# Procedure to analyse blood cytokine mRNA expression

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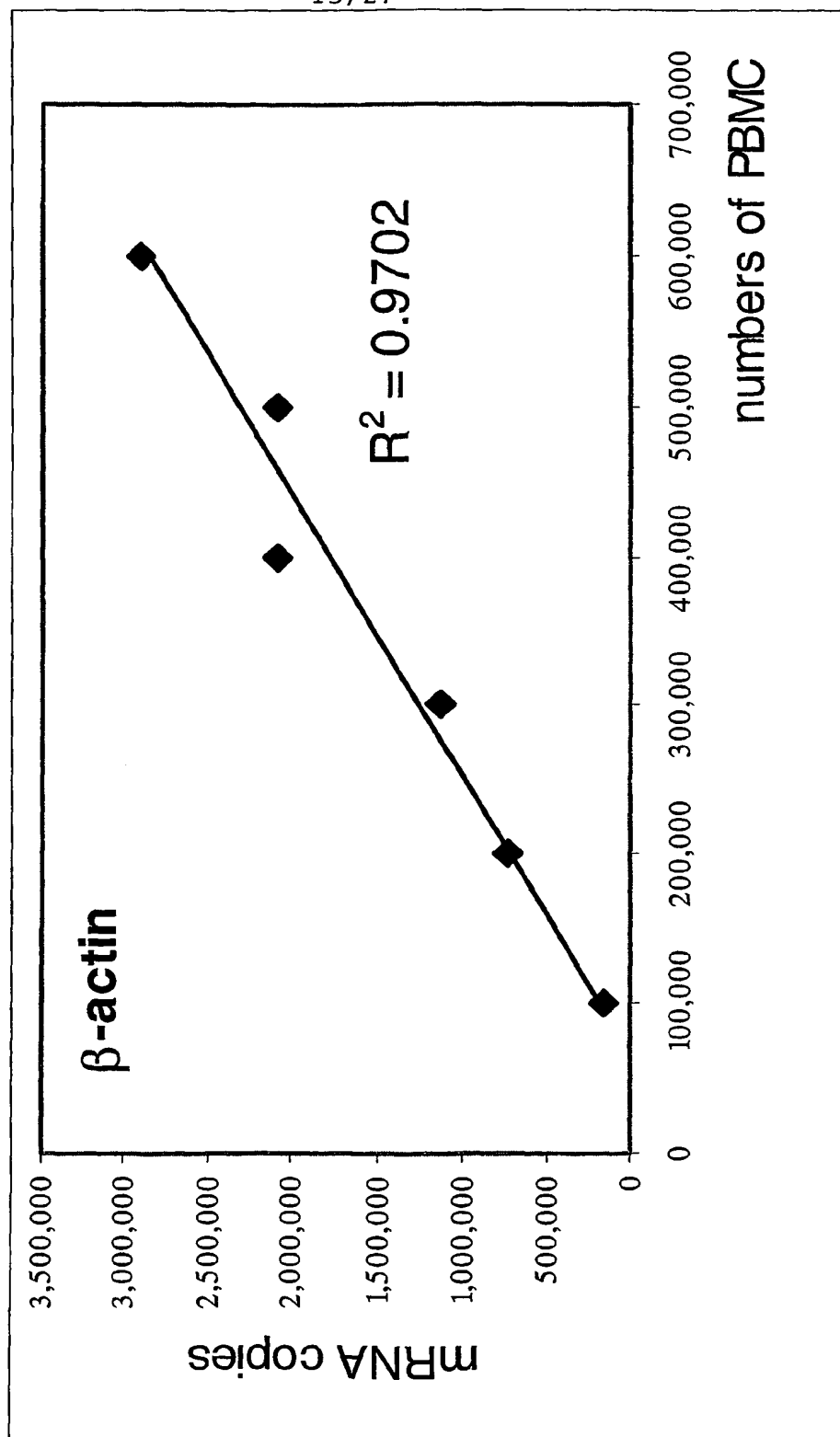
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- **Whole blood** sample incubation in the presence the tested potential immuno-modulator (antigen, allergen, cell, xenobiotic,...)
- Blood cells lysis and **mRNA stabilisation** using the reagent contained in the *PAXgene* tube
- **Automated** mRNA extraction and preparation of the reaction mixture for one-step RT-PCR using an automated device (*Magna Pure instrument – Roche Diagnostics*)
- Quantification of cytokine mRNA levels by **real-time PCR** using the *Lightcycler instrument (Roche Diagnostics)*

Figure 10

# Automated mRNA extraction and reagent mix preparation on the MagNA Pure : direct correlation between starting cell number and found copy number

Figure 11



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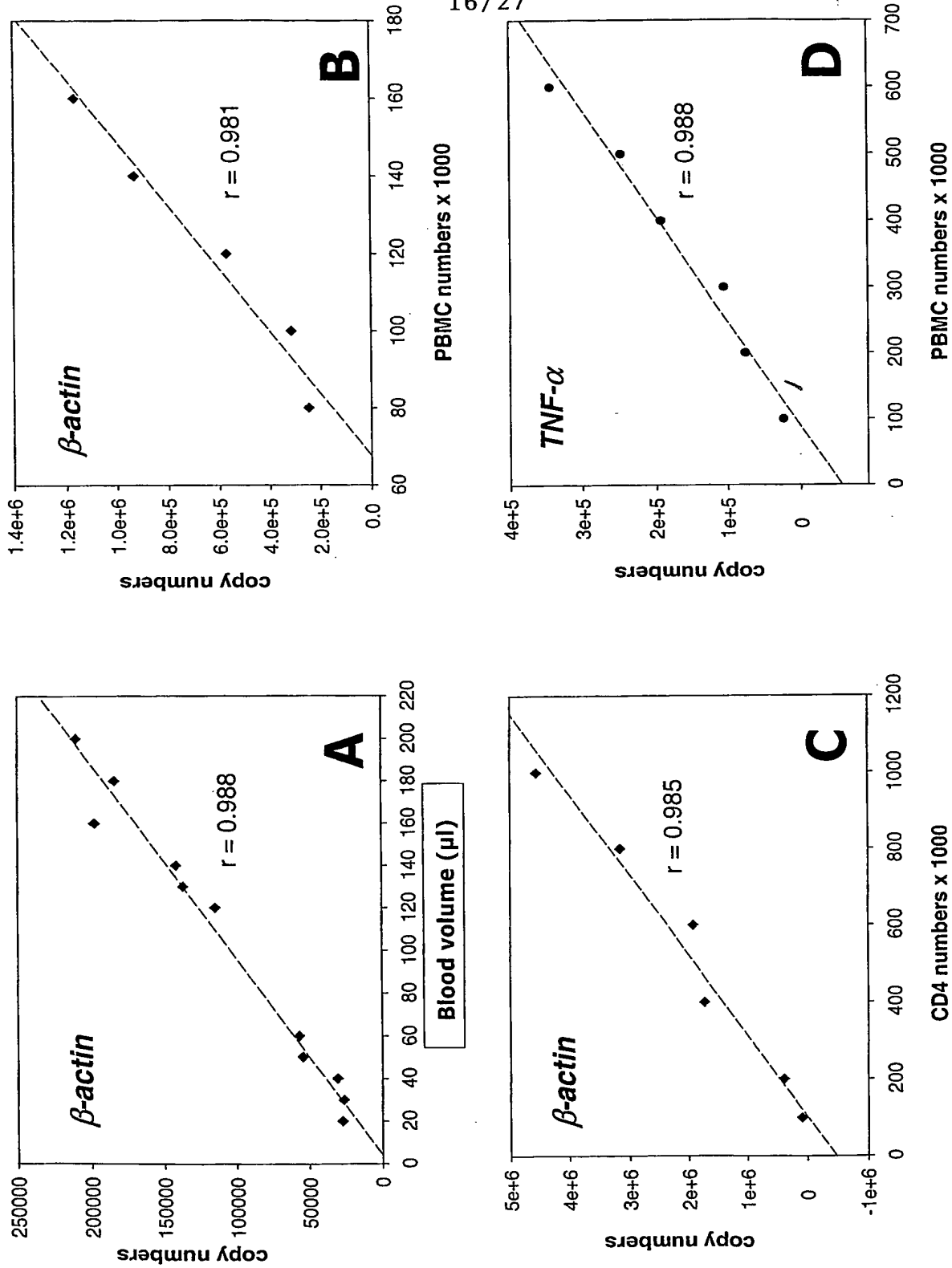


Figure 12



# Immune monitoring in cancer immunotherapy

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## Patient #3

- 7/99: melanoma of the right scapula. Excision
- 8/01: SC M+ arm, back, abdomen, right testis
- 4/02: right orchidectomy
- 4/02: pre-vaccine check-up
- 5/02: initiation of the vaccine program

Vaccine: MAGE-3 recombinant protein + adjuvant

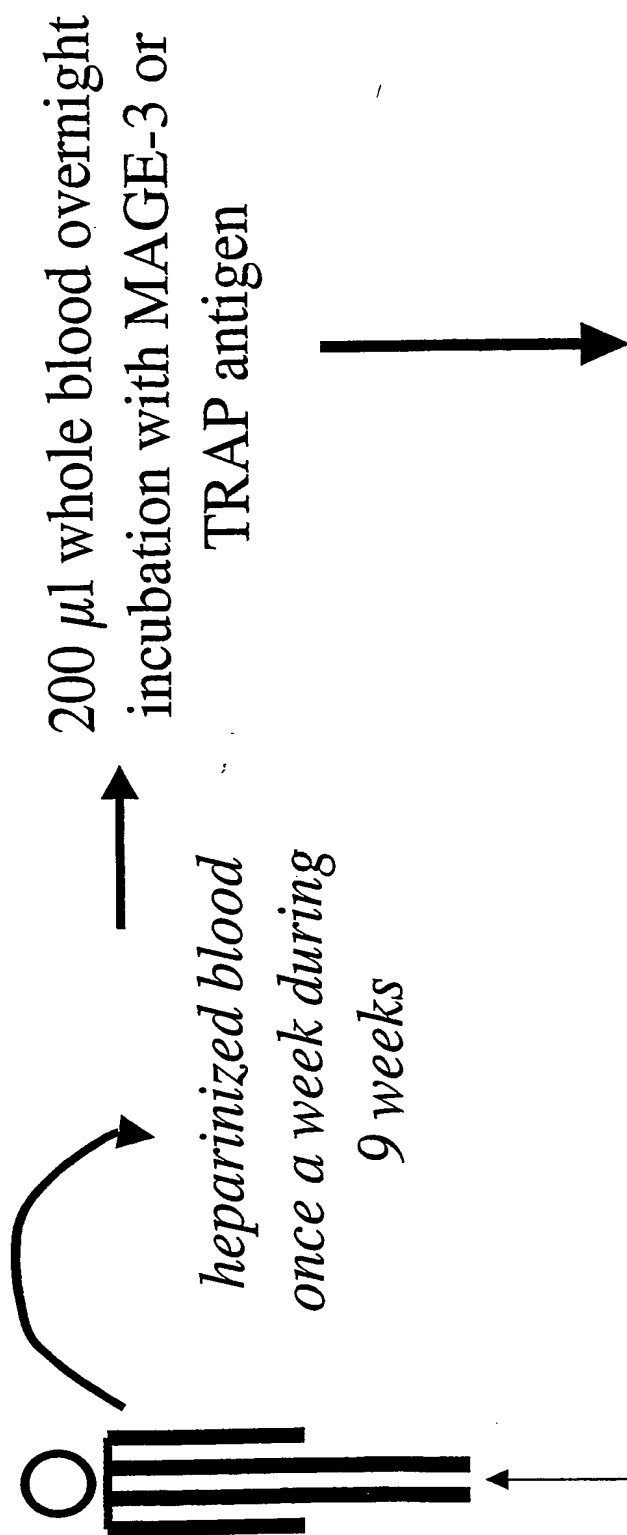
Figure 13

# *In vitro* stimulation of whole blood to assess immune response to MAGE-3

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Patient #3 received 3 injections  
of anti-MAGE-3 vaccine  
(MAGE-3 + adjuvant)

Figure 14

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# IL-2 mRNA in whole blood following MAGE-3 vaccination in Patient #3

## IL-2 mRNA

(copy numbers per million of  $\beta$ -actin mRNA copies)

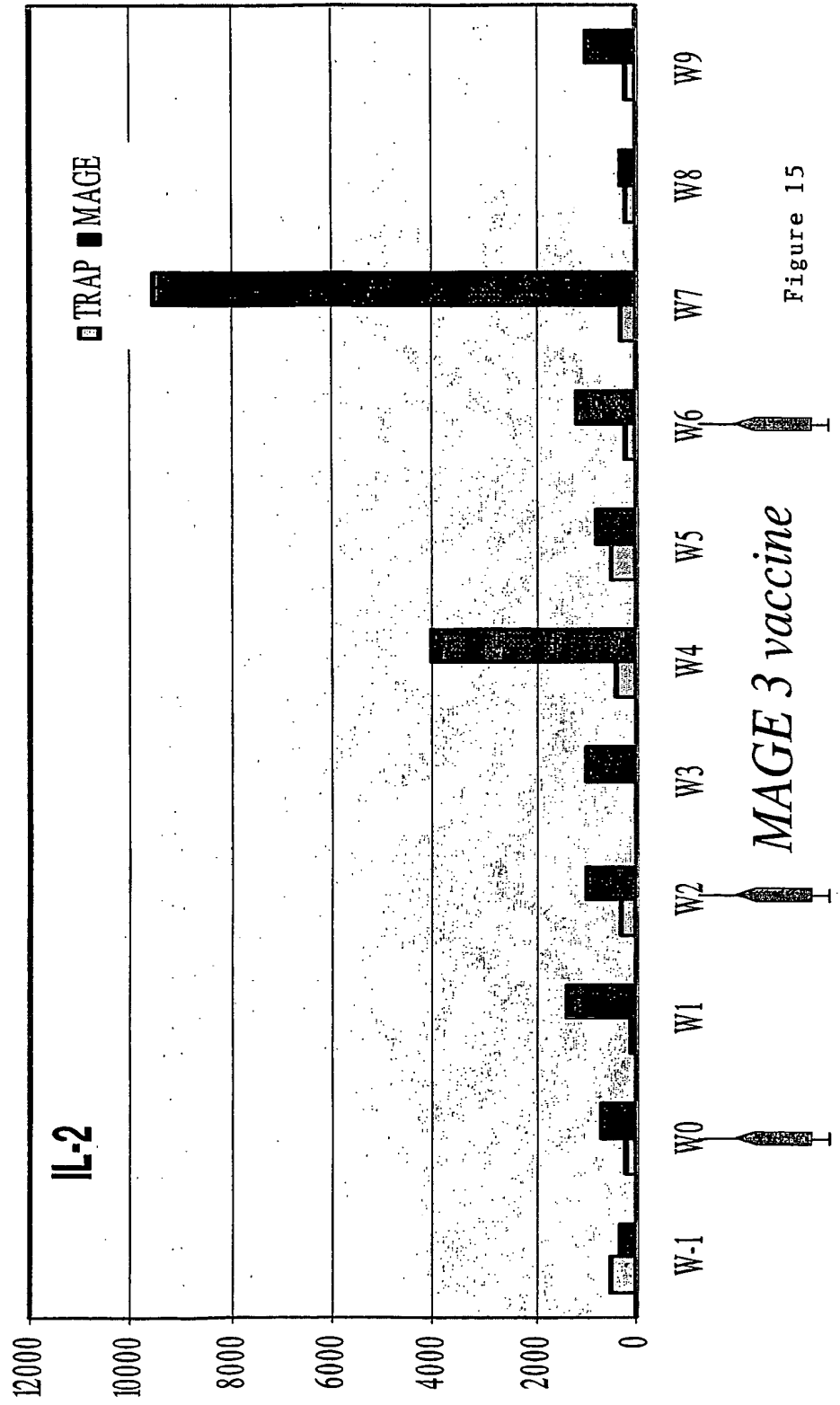
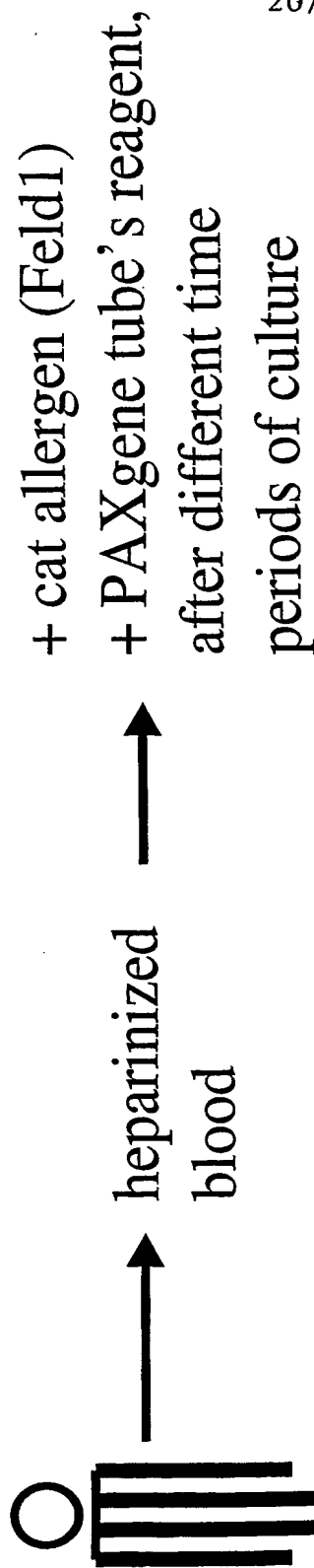


Figure 15

# *In vitro* stimulation of whole blood : evaluation of immune response to allergen

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mRNA extraction and RT-PCR  
using the MagNA Pure and the  
Lightcycler

Figure 16

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# *In vitro* stimulation of whole blood : evaluation of immune response to Feld1

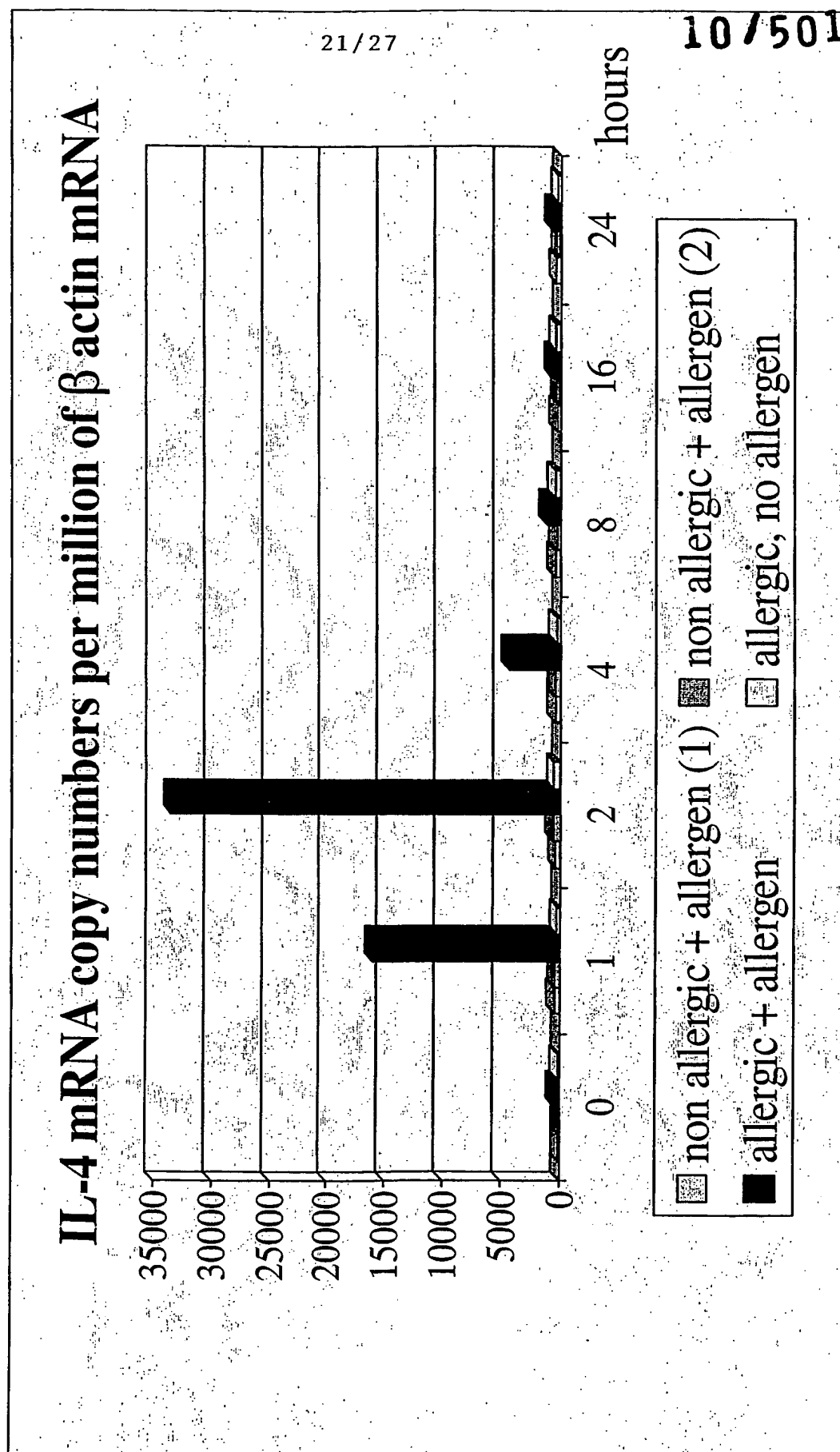
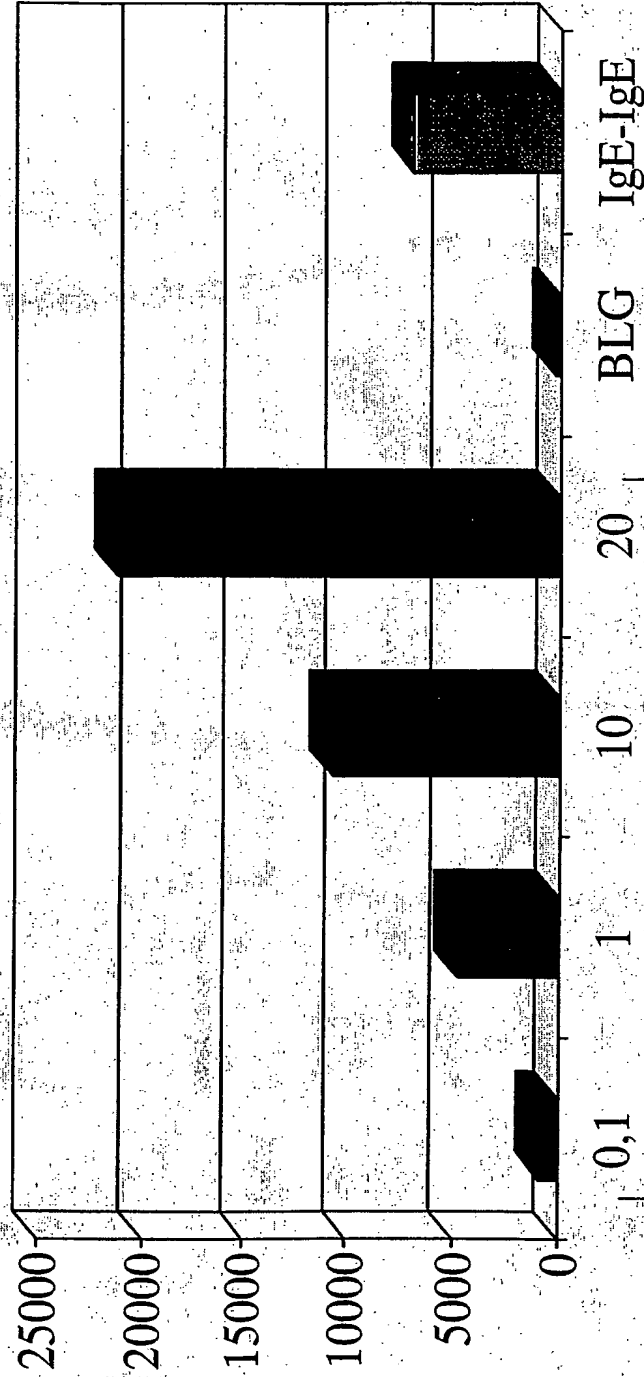


Figure 17

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# *In vitro* stimulation of whole blood : dose-response to Feld1

IL-4 mRNA copy numbers per million of  $\beta$  actin mRNA



$\mu$ g/ml Feld1 allergen  
(2 hours of culture)

Figure 18

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# IL-4 mRNA levels after whole blood stimulation with Feld1 are higher in allergic patients compared to healthy controls

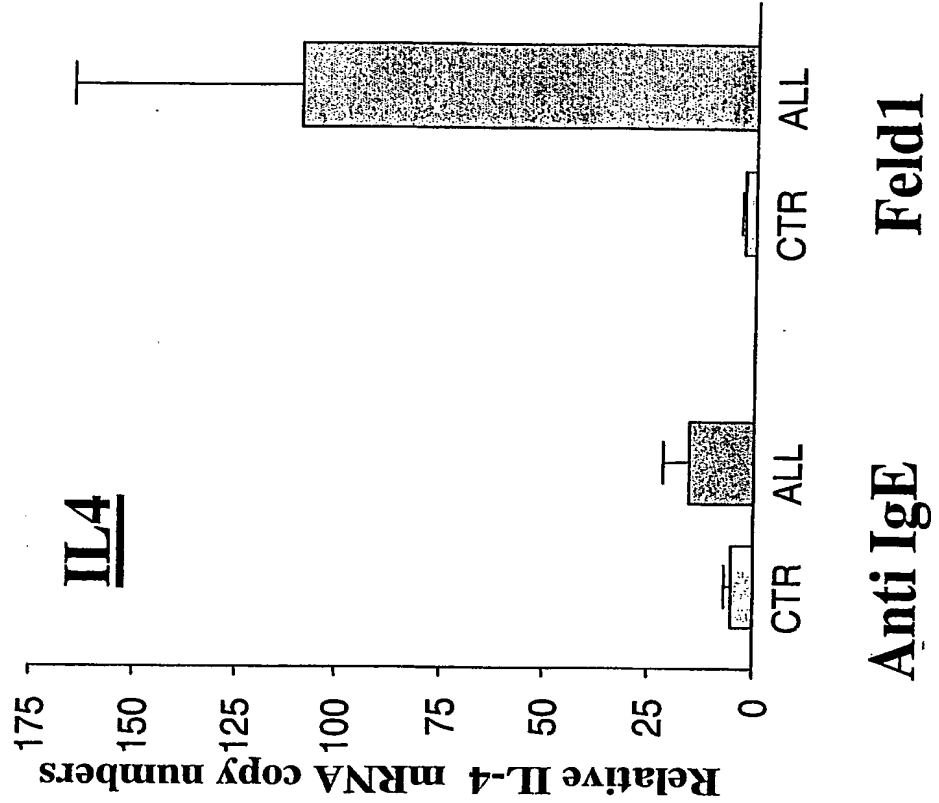


Figure 19

# *In vitro* stimulation of whole blood : assessment of T cell response to GAD65

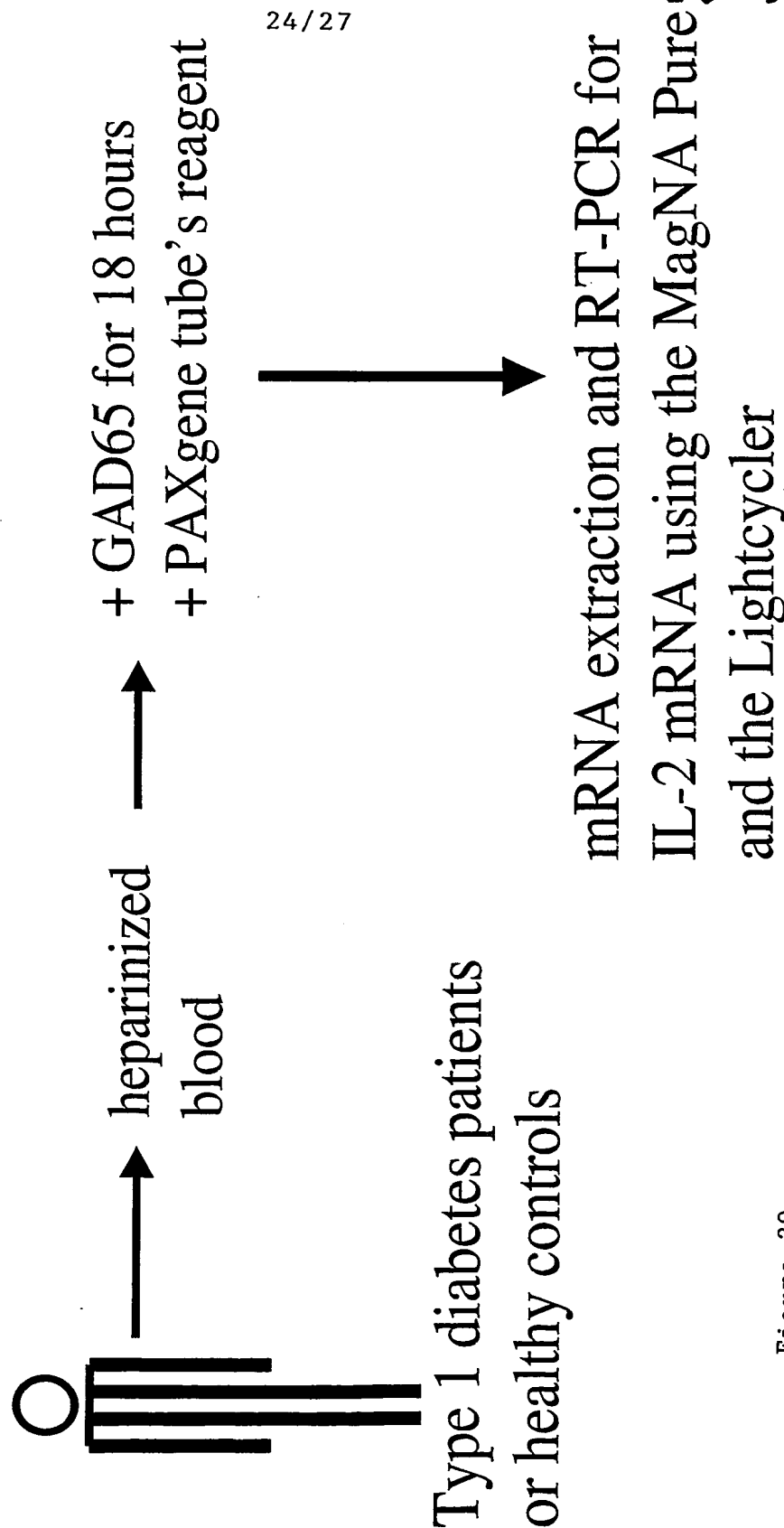


Figure 20



*In vitro* stimulation of whole blood :  
assessment of T cell response to GAD65

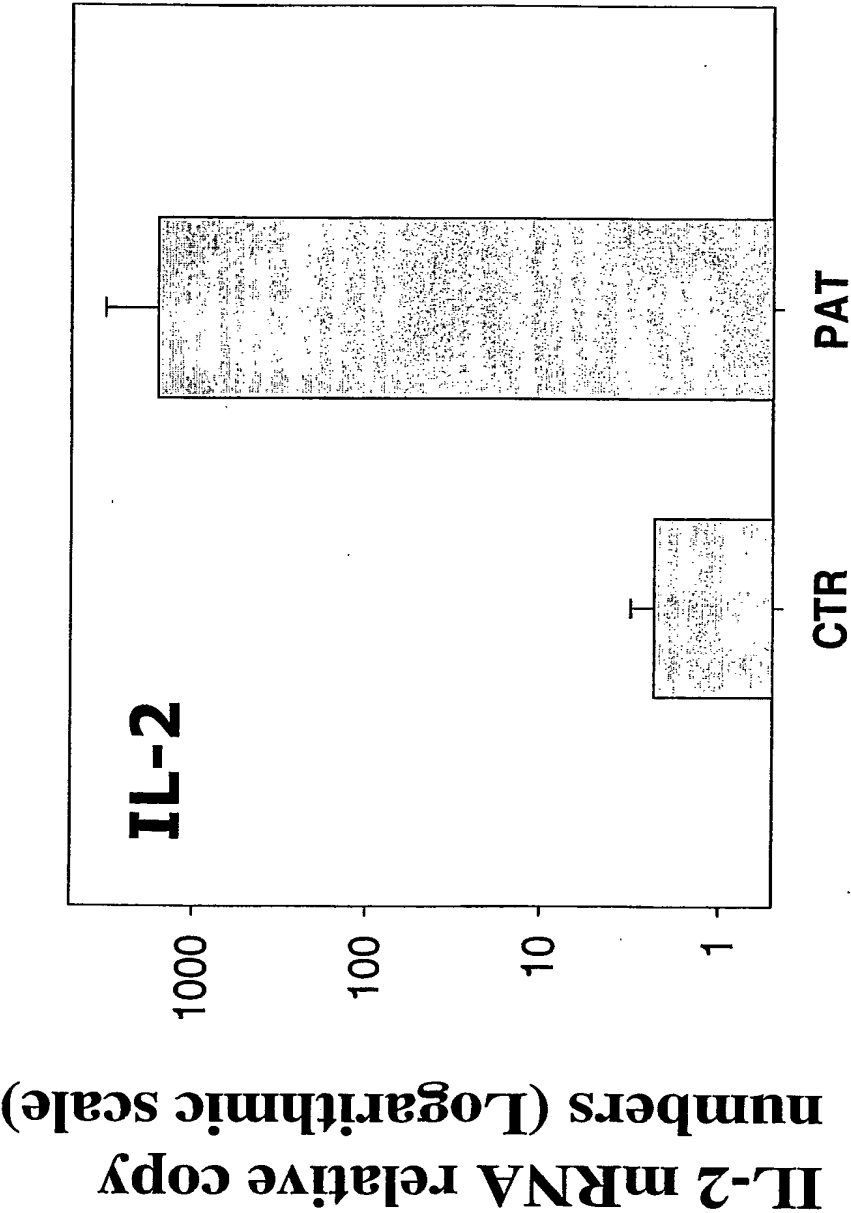


Figure 21

# Monitoring of alloreactive immune response : quantification of IL-2 mRNA in a whole blood + dendritic cells system

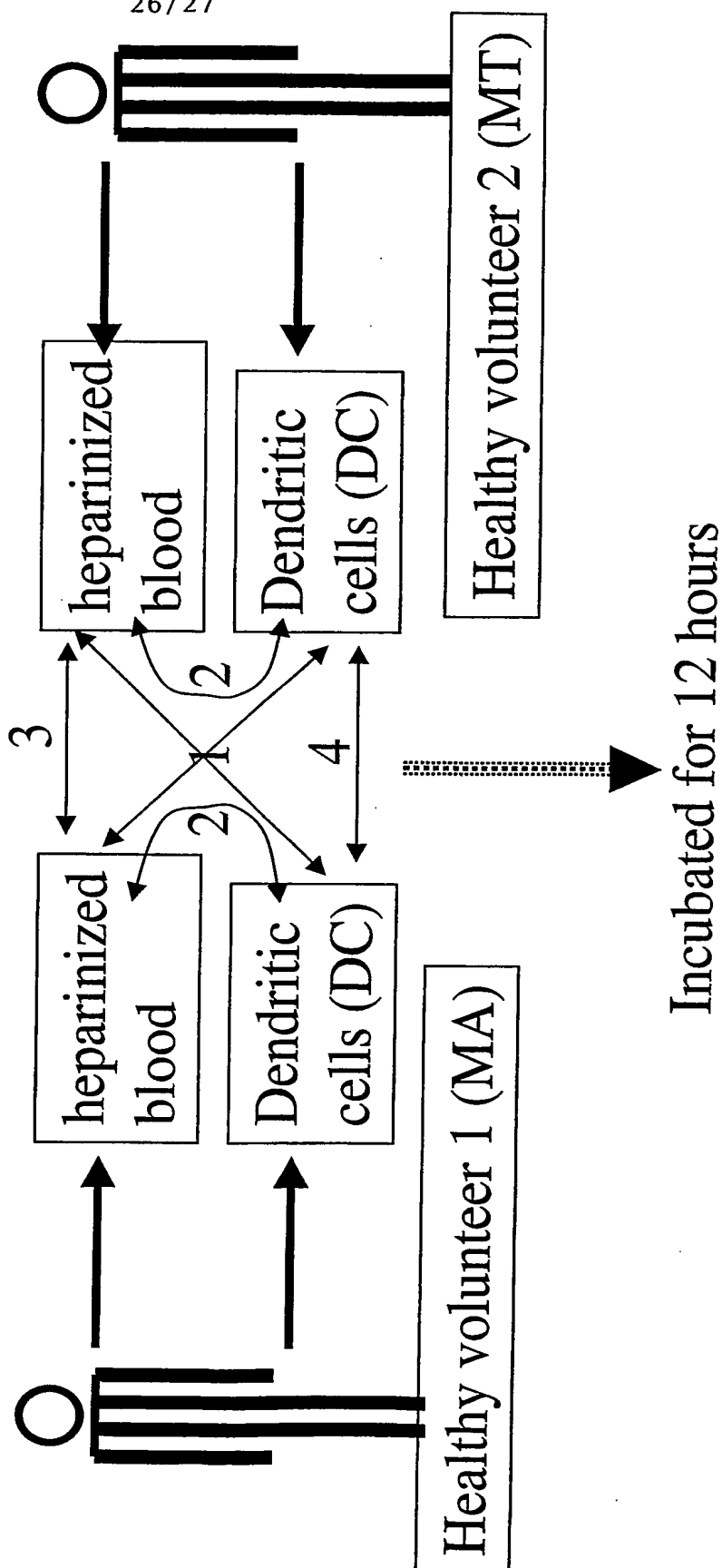
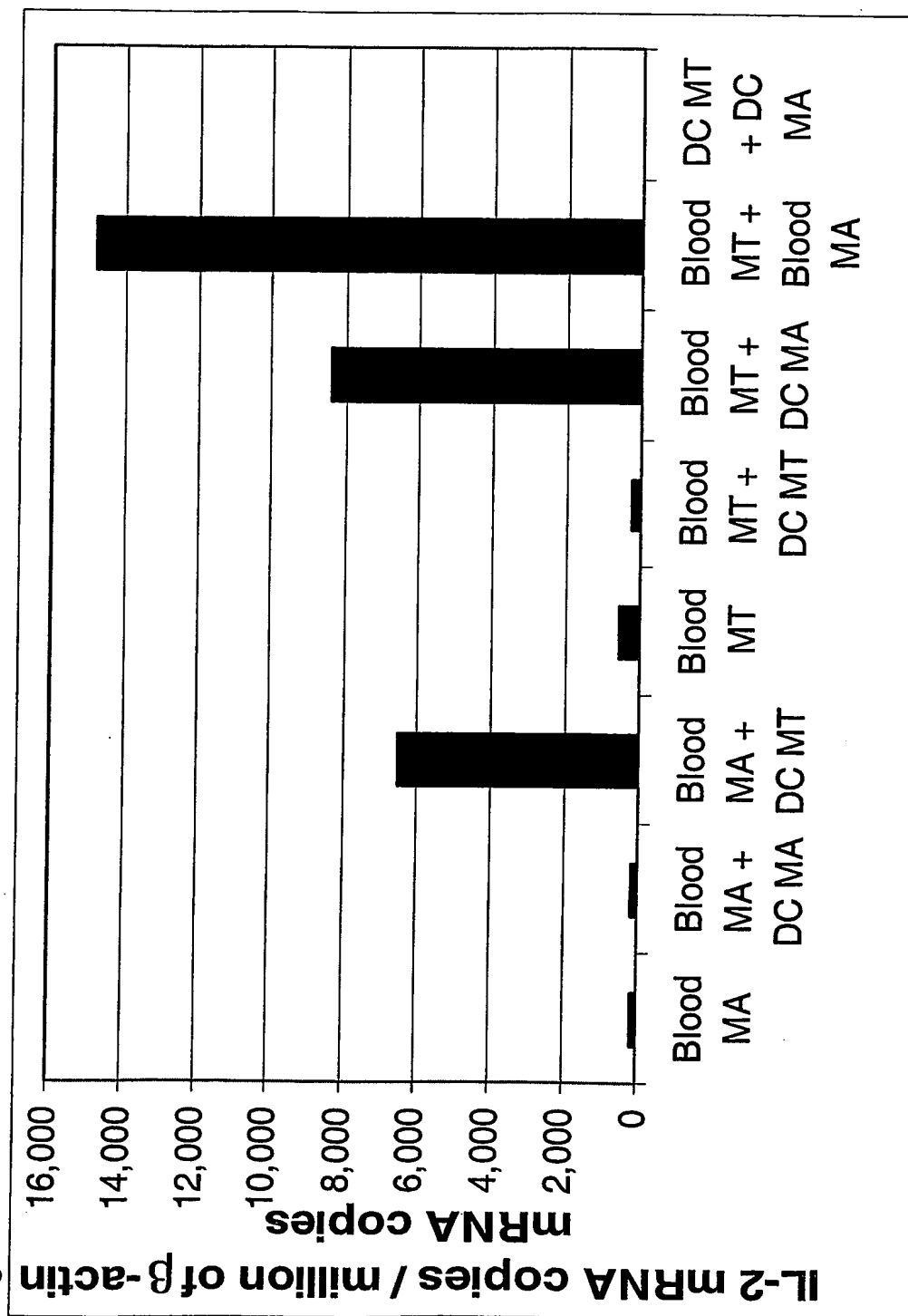


Figure 22

# Monitoring of alloreactive immune response : quantification of IL-2 mRNA in a whole blood + dendritic cells system

Figure 23



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